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Effects of Arsenic Cell Metabolism and Cell Proliferation: Cytogenetic and Biochemical Studies

by Johannes Petres,* Diethart Baron,† and Manfred Hagedorn*

Chromosome analysis of lymphocytes from patients who had been exposed to arsenic showed frequent structural and numerical aberrations, even with an interval of decades since the last exposure.

The *in vitro* addition of sodium arsenate induced the same chromosome changes–even to extreme of chromosome pulverizations–upon lymphocyte cultures from healthy subjects. Radioactive incorporation studies showed that arsenate was able to inhibit dose-dependently the incorporation of radioactively labeled nucleotide in RNA and DNA. Beyond that, arsenic blocked the cells in the S- and G_2-phase.

A general explanation for the inhibitory effect of inorganic arsenic on cell metabolism is the known strong affinity of arsenic to enzymes, especially to those containing sulfhydryl groups.

Introduction

The well-known carcinogenic effect of inorganic arsenic compounds indicates that these substances can directly or indirectly alter the distribution or composition of "genetic" material localized in the chromosome and thereby provide a prerequisite for the origin of atypical cell populations (*1*, *2*). We found massive lesions of the cellular nucleus—even "chromosome pulverizations"—after *in vitro* exposure to arsenic in lymphocyte cultures taken from subjects not exposed to arsenic (*3*). For this reason we studied the problem of whether the lymphocytes from persons who had previously come into contact with arsenic, either in their profession (vine-growers) or for therapeutic reasons (e.g., psoriasis) demonstrated corresponding chromosomal changes.

Supplementary experiments with *in vitro* administration of arsenate in cultured lymphocytes should explain to what extent these chromosome aberrations are dose-dependent and to what extent arsenic is capable of altering nucleic acid metabolism.

Materials and Methods

Cytogenetic Studies

Test subjects were 62 patients of the University of Freiburg (i. Br.) Dermatological Clinic. Of these patients, 31 had a history of extensive arsenic contact. In some cases, the last arsenic exposure was more than 30 years previously. The test group consisted of 14 psoriasis patients and 17 vine-growers. All displayed typical arsenic hyperkeratosis on the palms of their hands and the soles of their feet (*4*). Several of the patients already had arsenic-induced skin carcinomas excised. The control group (31 subjects) consisted of 14 psoriasis patients and 17 healthy volunteers without traceable history of arsenic contact.

For the chromosome analysis phytohemagglutinin (PHA)-stimulated lymphocyte cultures were prepared according to Moorhead et al. (*5*). Production of air-dried preparations was followed by staining with orcein diluted in acetic acid (*6*).

Radioactive Incorporation Test

The preparation of the PHA-stimulated lymphocyte cultures was carried out by use of the techniques described by Moorhead et al. (5). The prep-

*Department of Clinical and Experimental Dermatology, University of Freiburg i. Br., German Federal Republic.

†Institute of Biology II, University of Freiburg i. Br., German Federal Republic.

aration of the culture material for autoradiography and liquid scintillation counting has already been described in detail by Petres et al. (*7, 8*). To the individual lymphocyte cultures from volunteers not exposed to arsenic sodium arsenate was added in concentrations between 0.05 and 100 μg/ml culture medium 24 hr before stopping the culture. Cultures from the same subjects prepared in a parallel manner but without the addition of arsenic served as controls.

DNA Cytophotometry

Suspended lymphocytes exposed *in vitro* to sodium arsenate (0.1 to 500 μg Na_2HAsO_4/ml culture medium) were smeared on a microscopic slide, air-dried, and mounted. One series of preparations was stained with gallocyanin chromatalaun (*9*). Subsequently, the same preparation was covered with Kodak AR 10 stripping film and exposed for 10 days.

DNA in nonlabeled cells was determined cytophotometrically with an integrating microdensitometer (Barr and Stroud, Glasgow); measurement conditions: objective 100 ×, extinction level 0.75, magnification stage 5, wavelength 575 nm; 50 cells from each preparation were measured.

Results

Cytogenetic Studies

The frequency of chromosome aberrations among our subjects in the arsenic-exposed subject group was significantly above that of the controls. This difference is especially evident in chromatid and chromosome aberrations, which exhibit remarkably low spontaneous frequency (Table 1). The number of breaks per mitosis amounted to 0.07 in the arsenic-exposed group and only 0.002 in the controls.

Table 1. Chromosome aberrations in 31 chronic arsenic patients and 31 healthy subjects.

	No. of aberrations	
Type of abnormality	Arsenical exposure group	Control group
Secondary constrictions	52	13
Achromatic lesions	29	3
Gaps	58	9
Chromatid breaks	34	1
Acentric fragments	39	2
Dicentric chromosomes	3	0
Number of mitoses	1121	1247

In addition, structural, multiple numerical chromosome aberrations were found which differentiated the arsenic-exposed group from the controls. The aneuploidy is constantly caused by chromosome "deficiency" (Fig. 1). Our results confirm those of our preliminary studies in 1970 (*11*).

Radioactive Incorporation Test

The experiments show that even relatively low dosages of arsenic cause mild to severe impairment of nuclear division (Figs. 2 and 3). It is remarkable that even "pulverized" chromosomes are able to incorporate *in vitro* the radioactively labeled thymidine as precursor of DNA (Fig. 4).

Autoradiographic experiments have further shown that the incorporation of ^3H-labeled thymidine in the DNA above a dosage of 0.1 μg sodium arsenate/ml culture medium is reduced in dose dependency compared with the control preparations. The incorporation of ^3H-uridine in the RNA is less clearly inhibited by arsenic (Fig. 5).

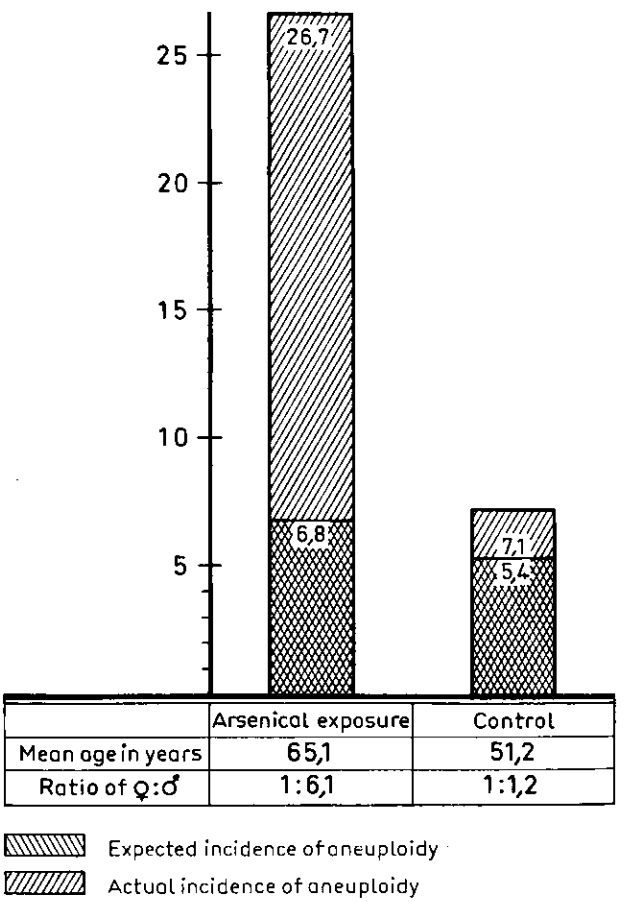

FIGURE 1. Frequency of aneuploidy mitosis (expressed as percent). Comparison of arsenic-exposed patients with the control subjects. Expected values from Court-Brown et al. (*10*).

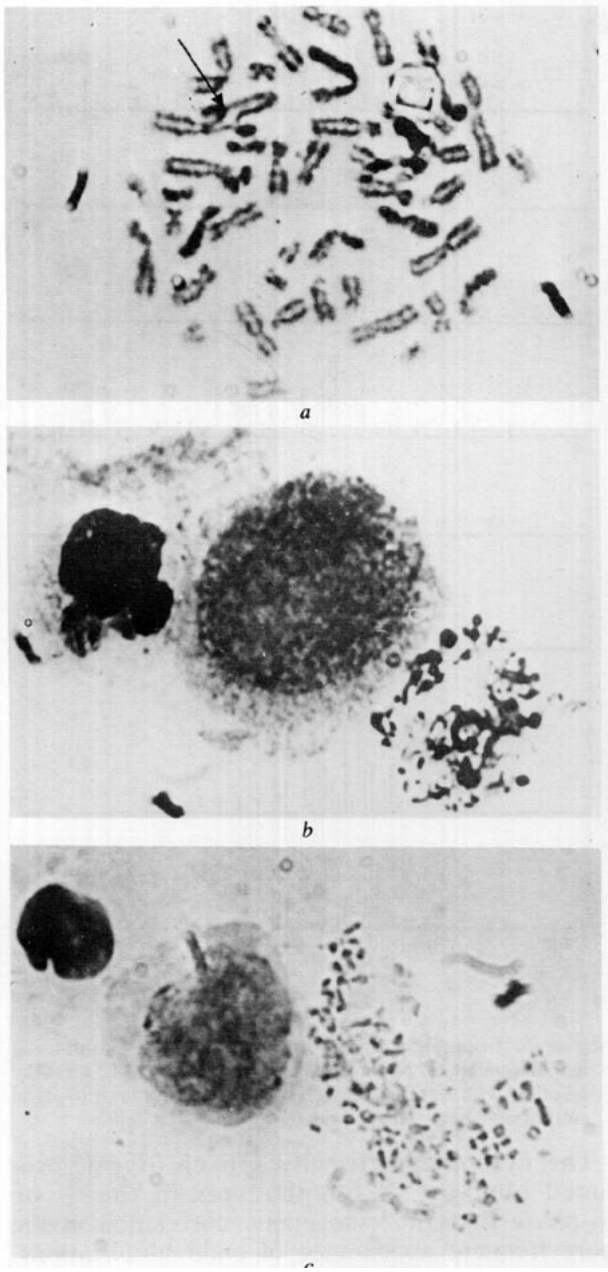

FIGURE 2. Preparations showing (*a*) metaphase of lymphocyte culture with 48-hr reaction with 0.1 μg Na_2HAsO_4/ml culture medium. (arrows show agglutination of two chromosomes); (*b*) metaphase of "arsenic-culture" (48-hr reaction with 1.0 μg Na_2HAsO_4/ml culture medium), showing severe nuclear division disturbance with blast-shaped transformed lymphocytes; (*c*) "arsenic culture" (48-hr reaction of 10.0 μg Na_2HAsO_4/ml culture medium) with two pulverized metaphases.

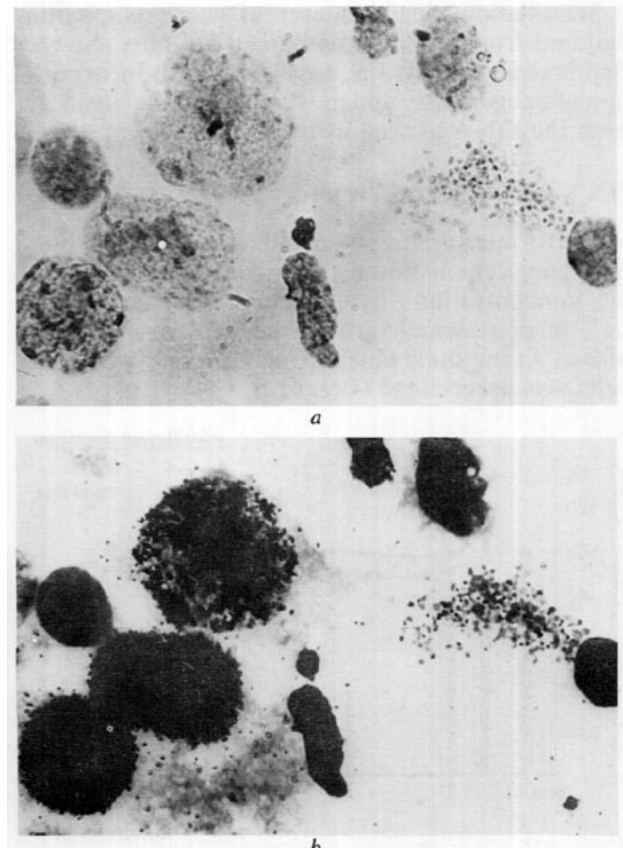

FIGURE 3. Preparations showing (*a*) metaphase of lymphocyte culture after reaction with arsenic (10.0 μg Na_2HAsO_4/ml culture medium) without stripping film; (*b*) slight tritium-thymidine-labeling of the above chromosome pulverization.

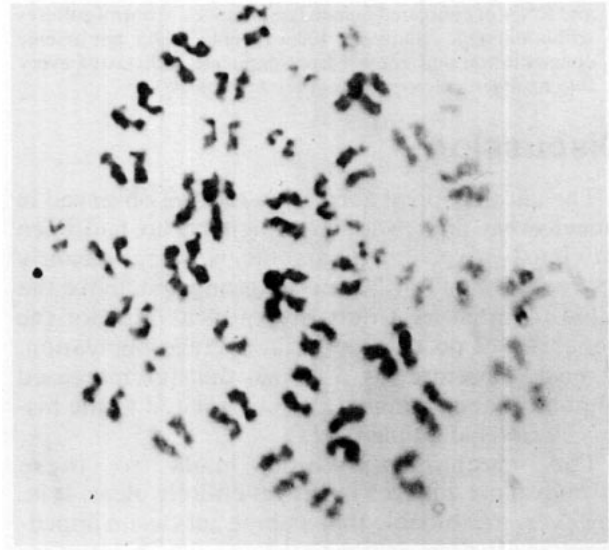

FIGURE 4. Metaphase of lymphocyte culture after 48-hr reaction of 1.0 μg Na_2HAsO_4/ml tetraploid set of chromosomes.

Scintillation measurements of acid-precipitating material from arsenic-treated cell cultures showed that arsenic inhibits the dose-dependent incorporation of exogenously added ^{14}C-labeled nucleoside in both the DNA as well as the RNA (Fig. 5).

DNA Cytophotometry

DNA measurements of PHA-stimulated lymphocytes demonstrated that under increased arsenic concentrations hyperdiploid cells decreased, i.e., in cells which are in the synthesis and G_2 phase. At the same time, an increase in hypodiploid cells was ascertained (Fig. 6).

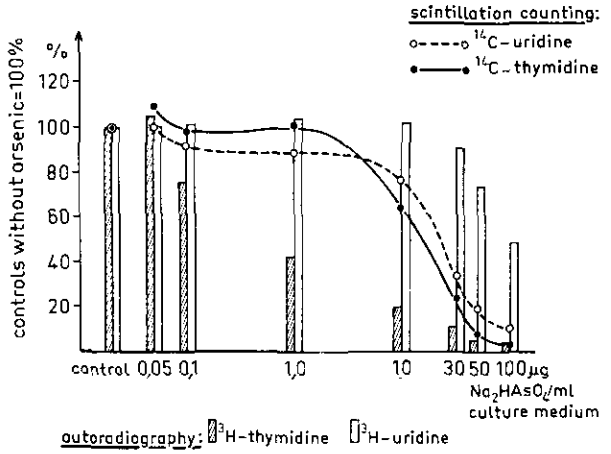

FIGURE 5. Graphic presentation of the influence of increasing Na_2HAsO_4 concentrations upon the incorporation of ^{14}C-thymidine and ^{14}C-uridine (scintillation measurements) and ^3H-thymidine + ^3H-uridine (autoradiography) in DNA and RNA of cultivated human lymphocytes. Control cultures without arsenic additive = 100% (average value per arsenic concentration and control from duplicate analysis of every five to seven cultures from different subjects).

Discussion

The chromosomal aberration rate we observed in vinegrowers and psoriasis patients who had been previously exposed to arsenic was significantly above the value for the control group and above the value reported by Court-Brown et al. (*10*) for the frequency of occurrence in an average population. It must, therefore, be assumed that the increased number of aberrations is directly related to the history of arsenic contacts (*11*).

The mechanism which leads to these chromosomal changes is not yet entirely clear. It is, however, presumable that arsenic acts as an impediment to diverse enzyme-systems which interfere with nucleic acid metabolism, especially by interaction with those enzymes which contain sulfhydryl groups (*8, 12–14*).

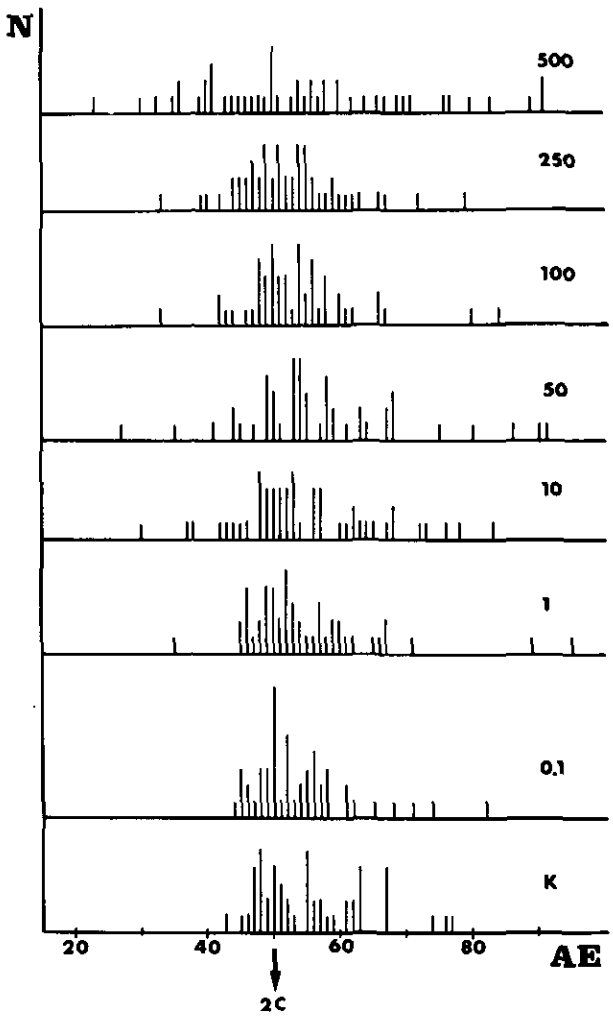

FIGURE 6. Frequency distribution of the DNA-containing RNA-stimulated lymphocytes in relative units (AE) after the reaction with various Na_2HAsO_4 concentrations (in µg/ml culture medium). Measurements of unlabeled cells.

The cytophotometrically proven arsenic-produced blocking of lymphocytes in the S- or G_2-phase finds its cytogenetic correlation in the more frequent appearance of endoreduplications, i.e., cells with tetraploid chromosome sets (Fig. 3).

A further site of activity by arsenic on cell metabolism could be that arsenic, as in the mechanism of decoupling of the substrate chain phosphorylation, either incorrectly builds into the nucleotide chains during nucleic acid synthesis or in higher concentrations competitively inhibits the incorporation of phosphorus. The inhibition would then be dependent upon the arsenic phosphorus ratio.

The results of our radioactive incorporation studies are comparable with those of Sibatani (*17*), who proved that inorganic arsenic compounds

could inhibit the incorporation of radioactive-labeled phosphorus into the nucleic acid. It is conceivable that the messenger RNA is already altered during its formation and delivers faulty information to the sites of protein synthesis, e.g., enzymes of replication, repair, and formation of precursors (*18–23*). Although not statistically significant, the different inhibition-curve progressions of thymidine and uridine incorporation indicate that the DNA-polymerase is more severely inhibited than the RNA-polymerase (*8*). These reactions could provide an explanation for the observed chromosome aberrations both *in vivo* and *in vitro* (*24, 25*). If primary cells are damaged as a result of contact to arsenic and subsequently survive, they could then become the stem cells (*26*) for a pathologic population still detectable many years later in the form of aneuploid cells or cells with marked chromosome anomalies in chronic arsenic intoxication (*11*).

This impairment mechanism has an effect not only upon lymphocytes but also other cell systems which in chronic arsenic patients manifest themselves in more frequent neoplasias of the skin, liver, and bronchi (*27, 28*). The few leukoses (*1*) can be explained by a lower sensitivity of the hematopoietic system to the mutagenic agent arsenic.

REFERENCES

1. Petres, J., and Hagedorn, M. Die Kaiserstuhl-Krankheit, ein Modell der chronischen Arsen-Intoxikation. Akt. Dermatol. 1: 177 (1975).
2. Jung, E. G., and Trachsel, B. Molekularbiologische Untersuchungen zur Arsencarcinogenese. Arch. Klin. Exp. Derm. 237: 819 (1970).
3. Peters, J., and M., Hundeiker. Chromosomenpulverisation nach Arseneinwirkung auf Zellkulturen *in vitro*. Arch. Klin Exp. Derm. 231: 366 (1968).
4. Hundeiker, M., and Petres, J. Morphogenese und Formenreichtum der arseninduzierten Präkanzerosen. Arch. Klin. Exp. Derm. 231: 355 (1968).
5. Moorhead, P. S., et al. Chromosome preparation of leucocytes cultured from human peripheral blood. Exp. Cell Res. 20: 613 (1960).
6. Schwarzacker, H. G., und Wolf, U. Methoden der medizinischen Cytogenetik. Springer, Berlin-Heidelberg-New York, 1970.
7. Petres, J., Baron, D., Kunick, I., and Rossner, R. Veränderungen der Nucleinsäure-Synthese durch Arsen bei isolierten menschlichen Lymphozyten unter Anwendung der Sedimentations- und Ficoll-Methode zur Zellgewinnung. Arch. Derm. Forsch. 250: 137 (1974).
8. Petres, J., Baron, D., and Kunick, I. Untersuchungen über arsenbedingte Veränderungen der Nukleinsäuresynthese *in vitro*. Derm. Mschr. 160: 724 (1974).
9. Sandritter, W., Kiefer, G., and Rick, W. Über die Stöchiometrie von Gallocyanin chromalaun mit Desoxyribonukleinsäure. Histochemie 3: 315 (1963).
10. Court-Brown, W. M., et al. Chromosome Studies on Adults (Eugenics Laboratory Memoir Series. XLII. The Galton Laboratory). Cambridge Univ. Press, London, 1966.
11. Petres, J., Schmid-Ullrich, K., and Wolf, U. Chromosomenaberrationen an menschlichen Lymphocyten bei chronischen Arsenschäden. Deut. Med. Wochenschr. 95: 79 (1970).
12. Margery, G. O., and Stocken, L. A. Biochemical effects of x-irradiation and the sulfhydryl hypothesis: a reappraisal. Nature 200: 136 (1963).
13. Stewart, T.H.M., et al. The effect of inorganic and organic arsenicals on the non-specific stimulation of lymphocytes by phytohemagglutinin in man. Proc. 5th Leucocyte Culture Conf. 1970, p. 75.
14. Petres, J., Baron, D., and Enderle, J. Zur stimulierenden Wirkung von Arsenat auf den Einbau von ^{14}C-Thymidin in die DNA Phytohämagglutinin-behandelter menschlicher Lymphocyten. Arch. Derm. Forsch. 251: 301 (1975).
15. Neubert, D. Einfluss von Pharmaka auf energieliefernde Reaktionen des Stoffwechsels. Arch. Exp. Pathol. Pharmacol., 246: 101 (1963).
16. Peters, R. A. Biochemical Lesions and Lethal Synthesis. Macmillan, New York, 1963.
17. Sibatani, A. *In vitro* incorporation of ^{32}P into nucleic acids of lymphatic cells. Effects of x-irradiation and some other agents. Exp. Cell Res. 17: 131 (1959).
18. Chargaff, E., and Davidson, J. N. The nucleid acids. Vol. I. Academic Press, New York, 1955.
19. Bollum, F. J., et al. Nucleic acid metabolism in regenerating rat liver. VIII. Effect of x-radiation on enzymes of DNA-synthesis. Cancer Res. 20: 138 (1960).
20. Hochster, R. M., and Quastel, J. H. Metabolic Inhibitors. Vol. II. Academic Press, New York, 1963.
21. Oppenheim, J. J., and Fischbein, W. N. Induction of chromosome breaks in cultures normal human leucocytes by potassium arsenite, hydroxyurea and related compounds. Cancer Res. 25: 980 (1965).
22. Jung, E. G., and Bay, F. Untersuchungen über "Dark-repair" Mechanismen menschlicher Epidermis. Arch. Klin. Exp. Derm. 235: 308 (1969).
23. Baron, D., et al. Further *in vitro* studies on the biochemistry of the inhibition of nucleic acid and protein synthesis induced by arsenic. Arch. Derm. Res. 253: 15 (1975).
24. Webb, J. L. Enzyme and Metabolic Inhibition. Vol. III. Academic Press, New York–London, 1966.
25. Frost, D. V. Arsenicals in biology—retrospect and prospect. Fed. Proc. 26: 194 (1967).
26. Seidel, A., and Sandritter, W. Cytophotometrische Messungen des DNA-Gehaltes eines Lungenadenoms und einer malignen Lungenadenomatose. Z. Krebsforsch. 65: 555 (1963).
27. Kuratsune, M., et al. Occupational lung cancer among copper smelters. Int. J. Cancer 13: 552 (1974).
28. Novey, H. S., and Martel, S. H. Asthma, arsenic and cancer. J. Allergy 44: 315 (1969).